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Eye Irritation Testing

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FOREWORD

During the past ten years the European Chemical Industry Ecology and Toxicology Centre (ECETOC) has published ten Monographs in which it has attempted to clarify, and express its views on, some of the more important problems in toxicology. This Monograph is a further addition to the series.

For many years the Draize rabbit eye test has been used to assess the potential damage to ocular tissue, following direct contact with chemical substances. The Draize test has received particular attention in the context of animal testing and consequently the development of alternative methods for testing is being sought. This Monograph assesses the current status of these developments and examines modified *in vivo* tests and alternative *in vitro* techniques in relation to their relevance to man, to the humane use of animals, and their utility for legislatory classification.

I therefore recommend this Monograph to all those who are concerned with both human and animal welfare.

R.R. Knowland
Chairman of ECETOC Board

A. SUMMARY AND CONCLUSIONS

The procedure described by Draize in 1944 has formed the basis of eye irritation testing for many years, and with some minor modifications has been adopted by various regulatory bodies for the assessment of the ocular hazard of chemicals and products.

In 1981 the OECD published Test Guideline 405 which harmonised test methodology. Eye tests carried out according to the guideline and their results are mutually acceptable to regulatory bodies within member countries of the OECD. Recently (1987) this guideline 405 was modified, primarily to reduce the numbers of animals used and to limit the occurrence of severe reactions. ECETOC fully supports this OECD initiative.

Since its introduction the Draize rabbit eye test has been criticised for a variety of reasons. These criticisms can be grouped under three headings : methodology, relevance of the animal model to man and the humane use of animals.

This monograph discusses the Draize method in detail and a number of conclusions are reached about specific aspects :

- a minimum number of animals (usually no more than 3) should be used;
- the routine use of extra animals to assess the effect of irrigation should be discouraged;
- there is a need to harmonise regulatory guidelines with respect to numbers of animals;
- the appropriate use of anaesthetics should be encouraged;
- any initial pain response at instillation is informative in relation to subsequent use of anaesthetics and to hazard evaluation;
- the more severe the irritant response initially the less important is the need to determine response reversibility.

There is limited information in the literature concerning the response of human eyes to chemical exposure. ECETOC attempted to obtain further information on human eye irritation from various sources. The information available suggests that the rabbit eye test may overestimate the ocular hazard to man in some cases but appears to be effective in demonstrating the absence of ocular hazard. There is a need to collect reliable human eye

irritation data if the effectiveness of the Draize rabbit eye test in predicting the effects in man is to be determined.

Three approaches were considered to reduce animal numbers and the potential discomfort of test animals : modification of the existing rabbit test, stepwise test programmes and the replacement of the rabbit eye test by alternative models. The Draize rabbit eye test is technically simple and modifications have been largely restricted to the reduction of dose volume and to dilution of the dose administered. ECETOC fully supports a stepwise test strategy which uses any relevant information that may eliminate the need for an animal test. Various in vitro, ex vivo and other alternatives are insufficiently validated to replace the rabbit model but some may be useful as screening techniques for inclusion in a stepwise strategy.

A number of systems exist for classifying ocular hazard on the basis of experimental data from rabbit eye tests. For a given chemical this can lead to a different classification of hazard based on the same experimental data and classification systems should thus be harmonised. Any change in test methods or the introduction of alternative assessments of ocular hazard will need to be considered in relation to the implementation of existing hazard classification schemes. It is also concluded that it is unimportant to distinguish experimentally in a rabbit eye test between a severe eye irritant and an eye corrosive chemical on the basis of the number of days taken to recover.

B. INTRODUCTION

The eye is one of the most valuable and vulnerable of the sense organs and any risk of disturbance of vision or of injury to the eye or even loss of sight from chemical or physical agents must be eliminated or minimised. Consequently, for many years the overall evaluation of the toxicological properties of chemical substances has included the assessment of their potential to damage ocular tissue following direct contact. This is a strict requirement of many national laws regulating the manufacture and use of chemical substances.

The Draize rabbit eye test, for determining ocular effects following the direct instillation of a chemical substance into the eye, was first published

in 1944. Since that time the test has been extensively used and forms the basis of all regulatory guidelines for the conduct of ocular irritancy tests. Although the Draize method has remained consistent, various approaches have been developed for the interpretation of data and the classification of chemical substances as irritant or not irritant to the eye.

The Draize rabbit eye test has been used by the toxicologist as a means of evaluating the potential of a chemical substance to damage the human eye. However, in the context of the debate on "alternatives to animal testing" and on the humane use of animals in scientific procedures, the test has received considerable attention and criticism from scientists, regulatory agencies, animal welfare groups and trade organisations.This in turn has stimulated the search for different ways of assessing ocular irritancy potential, including modification of the in vivo rabbit test and the use of in vitro techniques. In some countries strict guidelines have been introduced on the conduct of eye irritation testing in an attempt to optimise the balance between the need to safeguard the health of individual members of society and the welfare and humane treatment of laboratory animals.

The importance of this issue resulted in ECETOC's Scientific Committee setting up a Task Force to consider eye irritation testing using the following terms of reference :

1. To collect and assess all available data on results from protocols other than the standard OECD procedure for determining eye-irritancy of a range of chemicals and compare them with those obtained with OECD procedures.

2. To assess whether the use of results from these tests would lead to changes in the classification of chemicals as previously based on standard OECD protocols.

3. To assess the relevance of the experimental methods in predicting hazard to man, and whether these methods alleviate distress in animals and bring about a reduction in animal testing.

4. To state whether a ring test of the appropriate method(s) needs to be organised.

The above terms of reference capture the major points of debate regarding the Draize rabbit eye irritation test (human relevance, humane techniques, possible alternatives and classification of hazard). In addition, the terms of reference call for evaluation of novel tests and, if necessary, a proposal for a scientific evaluation of these tests.

Various factors influence the type of information that may be needed to establish the potential of any chemical to cause ocular damage. In particular the use of the substance (will it be used in close proximity to the eye ?) and the nature (voluntary or accidental) of the exposure are important factors. Some pharmaceutical preparations, contact lenses and their cleaning solutions are placed directly into the eye. Usually in these cases, eye testing is concerned mainly with demonstrating ocular tolerance, the materials being expected to cause no or negligible effects. For the majority of industrial, pesticide and consumer products any eye contact is usually undesirable and unintentional. For these chemicals the purpose of a Draize rabbit eye test is to demonstrate either the presence or absence (a knowledge of either is of equal importance) of any hazard of acute exposure.

This monograph addresses only the issue of eye studies for evaluating ocular hazard, and not ocular tolerance.

C. THE DRAIZE EYE TEST/METHODS

Due to the incidence of accidental eye injuries resulting from exposure to chemicals in the 1940s (Hughes, 1946; Temple, 1980) it became apparent that there was a need for methods to determine the potential eye hazard of chemicals and consumer products. The first reported experimental animal procedure was devised to investigate the effects of acids and bases on the eye and for the first time the effects on the cornea, conjunctiva and iris were separately recorded (Friedenwald et al., 1944). Carpenter and Smyth (1946) studied a wide range of chemicals, recording primarily effects on the cornea. Draize et al (1944), assessing the potential eye irritancy of drugs and other materials intended for use in and around the eye, standardised the method developed by Friedenwald and also simplified the scoring system.

In the Draize model the test material is instilled into the lower conjunctival sac of one eye of the rabbit. Any ocular response is assessed by observation of the eye for a period of up to 21 days after treatment. The

untreated eye is used as a reference control. The evaluation procedure developed by Draize subdivided the overall effect into three distinct components (corneal, iridial and conjunctival changes) and the magnitude of these effects was graded (Table 1). In this way, the "Draize" scoring scale evolved, and has since provided the basis of evaluation of eye effects.

Various regulatory bodies adopted and modified the basic Draize method for the assessment of potential eye hazard of household products and chemicals. The publication of the 1981 OECD Test Guideline (TG) - 405 achieved the harmonisation of the different existing eye testing methods. Data from eye tests carried out according to OECD TG - 405 should be mutually acceptable to regulatory bodies within the 24 Member countries of the OECD. In practice different regulatory bodies maintain different requirements, contrary to the spirit of the OECD Mutual Acceptance of Data agreement.

The OECD TG-405 has been updated and the essential elements of the revised OECD guideline (1987) are detailed in Appendix 1 in which procedures established by other legislative authorities are compared with that of OECD. The main differences are confined to the numbers of animals used and to methods of limiting the possibility of a severe ocular reaction.

In the following sections, the major aspects of the OECD guideline are considered and compared to guidelines issued by other regulatory bodies.

1. Experimental Animals

1.1. Selection of species

Albino rabbits have been used most widely in eye irritation studies because :

i) their eyes are relatively large compared with body size. Also the eye has a relatively large corneal surface area compared with the total surface area of the eyeball, and corneal changes are relatively easily observed (Swanston, 1985).

ii) the eyes are non-pigmented and therefore lesions in the iris are more easily observed (NAS, 1977).

iii) the rabbit eye presents a sensitive model for the detection of eye

irritants (Seifried, 1986). Lachrymal flow is low and viscous relative to man and materials instilled into the eye may be less readily rinsed out. In addition, the blink reflex is less well developed in the rabbit, which may result in a slower clearance from the eye compared with man. These factors may intensify contact and therefore may enhance the response compared with that likely in the human eye, thus providing an enhanced safety factor.

iv) Rabbits are relatively easily obtained and easy to maintain under laboratory conditions (Mc Donald and Shadduck, 1977; Swanston, 1983).

Other species have been considered as models for eye irritancy testing. Primate eyes are anatomically more similar to the human eye (Swanston, 1983) and limited trials have indicated that the monkey eye reflects more closely the human response than does the rabbit (Beckley, 1965-a; Beckley et al. 1969; Green et al., 1978; Buehler and Newmann, 1964; Freeberg et al., 1984). However the cost, availability and temperament of primates have limited their use in routine tests (Swanston, 1983). Nevertheless, the monkey has been recommended as the second test species when confirmation of rabbit data is required (NAS, 1977).

The dog has been used as an alternative test species in a few studies (Beckley, 1965-a; Giovacchini, 1972). The limited data available indicates that the eye of the dog is more susceptible to damage than that of the monkey and less susceptible than the rabbit (Beckley, 1965-a). Other non-primate laboratory species, such as the rat, guinea pig, cat and chicken have been considered as alternative test species but have not been fully evaluated (NAS, 1977; Seifried, 1986).

Human volunteers have been used in eye irritation tests but to determine the threshold of effect(s) rather than to identify the irritant potential of materials per se (Beckley, 1965-a; Beckley et al.,1969; Freeberg et al., 1986-a).

Inevitably, the species of choice for predictive eye testing represents a compromise between biological similarity to man and the practicalities of performing the test. On this basis, the rabbit has been chosen consistently as the test species and is the recommended species in all of the eye testing guidelines (cf Appendix 1). It must be recognised that the consistent use of

the rabbit in the Draize eye test procedure has resulted in a large reference data-base which is widely and regularly used.

1.2 Number of Animals

Regulatory authorities differ in their requirements for the number of animals per group in eye irritation tests.

Draize specified that 6 animals should be used, and this approach was adopted by FHSA (Federal Hazardous Substances Act) (1973), with the proviso that up to 12 more animals should be used if equivocal results are obtained. The methods proposed by AFNOR (Association Française de Normalisation) (1982), and the EPA/TSCA (1985)-FIFRA (1984) (Environmental Protection Agency/Toxic Substances Control Act/Federal Insecticide Fungicide and Rodenticide Act) also recommend the use of 6 animals per group. AFNOR and FHSA, advise that further animals should be used as required, while the EPA/TSCA-FIFRA suggest that fewer animals may be used if this can be justified (cf.Appendix 1).

Following harmonisation activities, OECD (1981) and EEC (1984-a) methods recommend a minimum of 3 animals with the provision that additional animals may be used in the event of equivocal results. The most recent OECD TG-405 (1987) recommends a "Single Animal Test" if marked effects are expected. If "severe" irritation or corrosion are observed, no further testing is needed unless it is necessary to investigate specific effects. If no severe effects are observed then two further animals may be treated. The UK Control of Pesticides Regulations (1986) advocate that only one animal should be used initially. In the event of a "strong" response the guideline indicates that the effects of irrigation should be investigated. If the material is not "strongly irritant", then 2 additional animals should be used.

1.3 Exclusions

Several of the test guidelines/procedures state that, in order to minimise the number of live animals used and the incidence of severe reactions, eye tests are unnecessary under certain conditions. These "exclusions", along with qualifications, are detailed below.

i) Physico-chemical properties and chemical reactivity. The physico-chemical properties and chemical reactivity of every test material/product should be considered prior to eye testing. pH is one of the important parameters to be considered and several guidelines, OECD (1981), EEC (1984) and EPA/TSCA (1985)-FIFRA (1984) suggest that because of their "probable corrosive properties" strongly acidic and strongly alkaline materials (pH < 2.0 and pH > 11.5) need not be tested.

ii) Results from dermal studies. OECD (1987), EEC (1984-a) and EPA/TSCA (1985)-FIFRA (1984) guidelines indicate that materials producing corrosion or severe irritation in skin studies need not be tested in the eye, it being considered that such substances will produce similarly severe effects on the eye.

iii) The use of in vitro alternatives. The 1987 OECD guideline acknowledges the contribution which in vitro procedures might play in avoiding unnecessary in vivo tests and suggest the use of well validated in vitro alternatives to identify severely irritant materials (see chapter E).

2. Test Conditions - Dose levels

All regulatory protocols call for the instillation of 0.1 g or 0.1 ml of the undiluted test material into the conjunctival sac of one eye of each test animal. For solids, the dose should not exceed the weight of substance occupying a volume of 0.1 ml or, if the material is particularly dense, a weight of 0.1 g. Test guidelines which consider the testing of aerosol products recommend that the aerosol is sprayed at the eye (which is held open) for approximately 1 second, and from a distance of 10 cm.

3. Procedure

3.1. The Use of Anaesthetics

The OECD (1987) guideline refers to the use of local anaesthetics in in vivo eye tests. The OECD recommends that anaesthetics may be used if it is thought that the test substance may cause "unreasonable pain" at the

time of instillation into the eye. The OECD points out that the anaesthetic, and its concentration should be carefully selected to ensure that its use will cause no significant differences in reaction to the test substance. In particular, anaestetic preparations with vasoconstrictor activity should not be used.

3.2. Irrigation

Washing is recommended by the OECD (1987) 24h after instillation of the test material and is carried out to remove any remaining test material from the conjunctival sac. Irrigation may be indicated for substances shown to be irritating. This is performed 30 seconds after the material has been applied to the eye and is continued for 30 seconds.

4. Clinical Observations and Scoring

All the guidelines use the "Draize" scoring scale as a basis to assess eye lesions (see Table 1). The 1987 OECD guideline states that eyes should be examined after 1, 24, 48 and 72 hours after treatment although it is common practice to observe animals more frequently, especially during the first 24 hours. Observations may be continued beyond 72 hours to assess progress of any lesion and its reversibility. This is formalised in some guidelines by asking for assessments after 7 and 21 days where necessary (see Chapter F). Continued assessment beyond day 21 is left to the discretion of the investigator. The OECD guideline also suggests that assessment may be aided by the use of various types of ophthalmological equipment and by the use of fluorescein dye to facilitate determination of any corneal involvement.

D. CRITIQUE OF PRESENT METHODS

The major criticisms of the Draize test which have been voiced over the years have been summarised by the EPA (Falahee et al., 1981)(see Table 2). The criticisms are broadly related to methodology, relevance to man and ethics.

In this chapter these criticisms will be discussed more fully, with those relating to methodology following the format of chapter C.

1. Comments on Methods.

1.1. Experimental animals

1.1.1. Selection of species. The sensitivity of the rabbit eye which was originally seen as a distinct advantage in the prediction of ocular hazard to man, is now a major source of criticism (Griffith et al., 1980; Sharpe, 1985). However, in a recent review which compared 7 animal models on the basis of anatomical, physiological and pharmacological factors, the rabbit was shown to be the most practical choice of species for eye irritation testing (Swanston, 1985).

1.1.2. Number of animals. Various publications have examined the influence of the number of animals on the assessment of the eye irritant potential of chemicals. De Sousa et al. (1984) investigated the effect of progressively reducing group size in eye studies on the "classification" of 67 petrochemicals . The results of the study indicated that a reduction in group number from 6 to 5 results in the same "classification" of 96% of the test substances, and a progressive reduction of group size to 2 resulted in the same "classification" of 88% of the test substances. These general findings were supported by Williams (1985) who also stated that the use of more than 6 animals per group was unnecessary. Both publications conclude that the smallest number of animals should be used which was necessary to provide the required level of precision in the test. This conclusion has been encompassed in the 1987 OECD TG-405 with the recommendation of a "single animal test" if marked effects are expected.

ECETOC fully supports the use of the minimum number of animals in eye tests but also recognises the need for the harmonisation of regulatory guidelines regarding the minimum number of animals required.

1.1.3. Exclusions.

i) Physico-chemical properties. pH alone cannot be considered as an indicator of potential eye irritancy. York et al. (1982) and Murphy et al.(1982) reported that materials with pH values outside the limits quoted in the TG can be tolerated in the rabbit eye. The recent OECD guideline (1987) states that buffer capacity (which

ECETOC interprets as the capacity for the chemical to maintain its own pH) should be considered as well as pH. Similar proposals have been made in relation to the skin toxicity of acidic and alkaline materials (Young et al., 1988).

ii) Results from dermal studies. The potential of a chemical to produce severe skin responses does not always correlate with the potential for severe eye irritation (Gilman et al., 1983; Williams, 1984; Rhodes et al., 1986).

In some cases, implementation of the above exclusions may result in

a) an assessment of chemicals as eye irritants when, in fact, animal tests may demonstrate the absence of significant ocular effects;
b) an inability to rank irritant and more severely irritant chemicals;
c) the erroneous classification of ocular hazard.

Nevertheless ECETOC supports the utilisation of the exclusions outlined above.

1.2. Test conditions

1.2.1. Dose levels

i) Dose Volume. The choice of 0.1 ml as the volume to be tested has frequently been questioned on the grounds that it greatly exceeds both the capacity of the rabbit eye and the quantity of material which may enter the human eye accidentally. The maximum volume of fluid retained in an unblinking rabbit eye is approximately 80 to 100µl (Swanston, 1985; Jacobs et al., 1987). By comparison the normal volume of fluid residing in the human eye is 10 to 30µl (Swanston, 1985; Jacobs et al. 1987). Therefore the use of a dose of 100 µl in a rabbit eye test represents a several fold excess when compared to human eye capacity.

Because of the interaction between pharmacological mediators of inflammation in the rabbit eye, the use of a 100 µl test volume may result in a response of greater intensity than would be seen in primates or man. This appreciation of the pharmacological sensitivity

of the rabbit eye has led Swanston (1985) to state that "over rigorous testing, which was characteristic of the original Draize approach, may raise the intensity of response to a level at which a self-perpetuating and atypical process is triggered, which maintains a prolonged manifestation of inflammation which is largely independent of the original chemical insult."

ii) Site of application. There has been some debate on the choice of the site of application of test substances (NAS, 1977). Instillation into the conjunctival sac may result in a longer exposure or contact time which in turn may produce a greater response when compared to the same material placed on the cornea, where it is likely to be swept away by the rabbit eyelids and which may be more similar to the accidental exposure of the human eye.

ECETOC's conclusion on the use of a lower volume in a Draize rabbit eye are recorded in Chapter E.

1.3. Procedure

1.3.1. The use of anaesthetics.

Opinions differ on the value of the use of corneal anaesthetics to reduce pain at the time of instillation of test materials into the eye. Johnson (1980) acknowledges the importance of the initial pain response in evaluating irritancy. Inhibition of the blink reflex at the time of instillation may prolong contact between the test material and the eye, and hence may increase irritancy. Arthur et al. (1986) reported "no meaningful effect on the course or intensity of response" following the use of anaesthetics. This view is supported by Heywood and James (1978) although they found some evidence of increased intensity of reaction when anaesthetics were used. Bell et al. (1979) reported that no increased level of response was observed in tests of dilute solutions of shampoos, although healing times were prolonged. Johnson (1980) and Ulsamer et al. (1977) indicated that the use of anaesthetics resulted in an increased irritant effect in some cases. There are no reports in the literature of corneal anaesthetics reducing irritancy.

In the opinion of ECETOC the use of anaesthetics should be considered in any circumstances where alleviation of any unnecessary pain or distress at the time of instillation can be achieved.

1.3.2. Irrigation. Rinsing of the eye was not considered by Draize et al. (1944) but has subsequently featured as an option in most regulatory protocols. The objective of irrigation soon after dosing and/or washing 24 hours after dosing has never been clearly enunciated.

It is assumed that the purpose of irrigation soon after instillation is to remove test substance from the eye in order to establish the effect on the nature and duration of any eye response. This information is assumed to be helpful in assessing the effectiveness of eye irrigation as a remedial measure.

It is assumed that washing the eye after 24 hours is to remove residual test material and to minimise the possibility that it would contribute to irritation persisting throughout the observation period.

No explanation of a need for irrigation is included in official test methods (e.g. EEC, 1984-a; OECD,1987). The effect of irrigation on the development of an irritant response is influenced by various factors, e.g. time after instillation of test substance, volume of rinsing solutions and duration of rinsing procedure.

Draize (1959) recommended irrigation 2 or 4 seconds after instillation. This is impracticable and seems too short an exposure, if the intention was to simulate rinsing after accidental eye injury. Seabaugh et al. (1976) studied the effects of irrigation on the irritant response to chemicals , and reported that in over 70% of the materials studied, the irritation response remained unchanged following irrigation either 30 seconds, 2 minutes, or 24 hours after instillation. Rinsing may have little effect on irritation unless it is performed within 10 seconds of instillation of the test material. (Davies et al., 1976).

In considering the value of irrigation, the NAS (1977) concluded that "the variability of irrigation techniques and the arbitrary nature of

any one regimen further complicates a complex test without providing much useful information. For these reasons, irrigation is not a recommended requirement for any test for the inherent irritancy of a substance".

Various regulatory guidelines indicate the need to test the effect of irrigation on the development of an ocular irritation response. However, the results of these studies are not considered in any hazard classification and rarely influence the standard first aid practice of extensive irrigation of the eye followed by appropriate medical treatment.

Any effect of irrigation needs to be assessed in relation to the irritant effect of the test substance without irrigation. Consequently more animals are required. ECETOC believes it is questionable whether the information derived from routine irrigation studies justifies the increased number of animals used.

1.3.3. Clinical observations and scoring. In relation to the Draize scoring scale Ballantyne and Swanston (1977) commented that : (i) focussing on selected effects on three tissues (cornea, conjunctivae, iris) may give an incomplete picture of effects on the eye in toto, (ii) effects on tissues considered less important are under-emphasised or negated, (iii) results are reported as weighted arithmetic scores rather than grades of effects.

Subjective scoring of eye lesions is one of the difficulties associated with the Draize test (Weil and Scala, 1971). Interlaboratory variation in subjective assessment has been thoroughly studied. The EPA (Falahee et al., 1981) commented that "scoring systems based on subjective examination generally are considered adequate although ... the use of these systems had led to inaccuracies in the assessment of eye irritants."

The FDA (1965) and the CPSC (1976) have published illustrated guides to aid the consistency of scoring.

Various techniques have been recommended to facilitate and supplement the Draize evaluation of eye irritation. Parameters investigated

include corneal thickness, intraocular pressure, corneal and conjunctival weight, capillary permeability and histological examination (Falahee et al., 1981).

ECETOC concludes that while some of these measures may allow standardisation of techniques and objective measurement, they may not always be appropriate to the overall assessment of the development of, and recovery from an ocular lesion.

1.3.4. Initial pain response. An integral part of the rabbit eye test is the assessment of the "initial pain response" upon instillation of a test substance into the eye. Assessment is subjective and whether the response is negligible or substantial is obviously relevant to the evaluation of the overall hazard. This information is however not required by any regulatory procedure. There may be no correlation between the potential for causing an initial pain response and subsequent irritation (Price, 1987).

ECETOC concludes that, the consideration of the initial pain response may aid the interpretation of test data and the assessment of hazard.

1.3.5. Recovery of the eye response. Because of the possibility of permanent injury to the human eye, information on the extent of the recovery from any damage may be as important as a knowledge of the degree or nature of a lesion. The determination of reversibility of a lesion requires the prolongation of an experiment, in some cases for several weeks. In humane terms the determination of response reversibility in a rabbit eye test is contentious since it is associated with the presence of severe local lesions in the eye for the duration of the observation period. However, Morgan et al. (1987) have reported recently that corneal pachymetry performed 3 days after chemical application is predictive of the eye irritation classification determined by observing the ocular response for 21 days.

ECETOC considers that, for chemicals which initially cause "severe" local lesions in the rabbit eye, a knowledge of lesion reversibility is of relatively minor importance because a human eye exposed to such a material would normally be treated immediately and symptomatically. A variety of factors, which are difficult to simulate in a rabbit eye

test, may influence the prognosis of severe lesions in man e.g. speed and quality of initial treatment, concurrent exposure factors (e.g. other chemicals, temperature), presence of bacterial infection, standard of personal hygiene etc.

Conversely, for the purpose of hazard evaluation it may be important to determine the persistence of lesions which are of moderate or less intensity in a rabbit eye test. "Moderate" lesions, e.g. as defined in the NAS scheme (see Chapter F), would not normally be associated with distress in an animal test and the vast majority of these lesions will regress within the first 7 days of an experiment. However if lesions of this intensity persist then the chemical may be considered a greater hazard.

2. Relevance to Man

The ability of the Draize rabbit eye test to predict human ocular hazard has been criticised on the basis of biological differences between species and the volume used in the test. Both factors, which indicate that the rabbit test will over-estimate the hazard to man, are discussed above. For a final analysis comparative data on the response of the human and rabbit eye is essential, but unfortunately, there is a scarcity of human data on chemically induced eye irritancy in the open literature, most papers giving only a brief, unsubstantiated statement on the human response (Grant, 1974; Patty, 1981). Eye irritation tests on man have been limited for ethical reasons but a carefully controlled test has recently been reported by Freeberg et al. (1986-b).

In an attempt to establish the available data base on human eye incidents involving chemicals, ECETOC contacted various UK eye hospitals and a number of Poison Control Centres within Europe. No relevant data were readily available from any of these sources. In some cases this reflected problems of accessibility to stored information rather than the absence of case histories.

Other potential information sources investigated by ECETOC were the literature on chemicals which appear in official lists as "dangerous" on the basis of irritant potential to the human eye, the occupational

health department records of member companies and the published results of consumer surveys of surfactant based products.

2.1. Chemicals classified as eye irritants

Those chemicals included in the EEC Directive 67/548/EEC - Annex 1 with risk and safety phrases, R34, R35, R36, R36/37, R37/38, R36/37/38, S24/25, S25, S26, all of which identify hazard to eyes (R34 and R35 specify "causes (severe) burns" and are not specific to the eye) were examined. In total 219 chemicals were listed but a literature search revealed information on the human ocular irritancy of only 20 of these chemicals (Grant, 1974).

2.2. Occupational health data

From the medical departments from some member companies ECETOC obtained data relating to eye accidents involving chemicals which had occurred during manufacturing operations (see Appendix 2). The vast majority of incidents did not appear to lead to effects which persisted for longer than one to three days after the accident. The exceptions to this general rule were incidents involving chemicals of particularly low (acid) or high (alkaline) pH. The response seen in man was less severe than would have been expected on the basis of the animal data. However the dissimilarities of exposure, particularly in relation to precautionary measures and first aid and medical attention in the human cases, could not be taken into account when comparing the human and animal data base.

2.3. Preparations involved in human eye incidents

Investigations of the time for the human eye to recover from 515 eye incidents, involving consumers and manufacturing employees accidentally exposed to detergent products, indicated that 88% of eyes had "cleared " in 4 days or less (Freeberg et al., 1984 (1981-2 data)). A report of a further study (1983-4)involving 381 reported cases showed that 90% of the eyes had returned to normal in 1984 within one day, with an overall average recovery time of half a day (Freeberg et al., 1986-a). With the same products the irritation was

less persistent than seen in the Draize test; 20 to 30% of rabbit eyes had not returned to normal after 5 weeks.

2.4. General comments on relevance to man

Based on the limited human eye data available, the above information suggests that the Draize rabbit eye test may over-estimate the ocular hazard of chemicals to man. The differences in response of the rabbit eye compared with the human eye are probably due to the greater sensitivity of the rabbit eye, differences in exposure volume and to the fact that the rabbit eye, contrary to normal practice in man, does not receive any remedial treatment. On the other hand, the increased awareness raised by the Draize rabbit eye test of the likely effects of ocular exposure of some chemicals has undoubtedly reduced the number of human accidents. ECETOC has found no evidence from the above data of chemicals which were significantly irritant to the human eye but were not irritant in the Draize rabbit eye test. Therefore it would appear that the Draize rabbit eye test is useful in demonstrating the absence of any ocular hazard. This is significant since the majority of chemicals tested (approx. 65%) are categorised as being of no hazard to the eye (Kobel and Gfeller, 1985; Rhodes et al., 1986).

ECETOC considers that there is a need for the collection and review of human data relating to eye irritancy due to chemical exposure.

3. Humane Considerations.

Ethical considerations have been the main theme of public and media debate in criticising the Draize rabbit eye test. The principle concerns are : the use of animals, the number of rabbits used, and the extent of pain and distress which may be involved. These aspects are addressed on a number of occasions throughout this report.

E. ALTERNATIVE APPROACHES TO THE ASSESSMENT OF EYE IRRITATION

In view of the criticisms of the Draize rabbit eye test a number of alternative approaches to the assessment of the eye irritancy potential of chemicals have been proposed and are considered here under three categories :

1) Modification of existing techniques;
2) Replacement by alternative test systems;
3) Use of stepwise programmes.

1. Modification of Existing Techniques

1.1. OECD Test Guideline-405.

The updated OECD guideline (OECD, 1987) incorporates modifications to reduce the need for unnecessary testing (see Appendix 1). A new section "Initial Considerations" has been included which stresses the importance of minimising the testing of materials likely to be severe eye irritants. It includes the concept of using "well validated alternative techniques" as a pre-screen, although "well validated" is not defined. The updated guideline also introduces the idea of a test on a single animal if marked effects are anticipated. If a severe effect occurs further testing may not be necessary, thus reducing the number of animals to be used.

Two changes have been made to that part of the guideline which deals with irrigation. The option to irrigate eyes 4 seconds after application of the test substance is considered impracticable and has been deleted. The recommended duration of irrigation is reduced from 5 to 0.5 mins and this would decrease the degree and the duration of any discomfort associated with irrigation. The overall result is that the number of animals used for irrigation studies is reduced from 6 to 3.

ECETOC endorses the OECD initiative which may reduce the number of animals tested as well as the possibility of the occurrence of severe responses.

1.2. Low volume technique.

Consideration of physiological factors suggests that the instillation of 0,1 ml of test material into the rabbit eye will lead to an overestimation of the human hazard (see Chapter B). Griffith et al. (1980) compared the human hazard reported in Grant (1974) to the response in rabbit eye tests of 21 chemicals which included surfactants, acids and alkalies. They concluded that a dose volume of 0.01 ml more accurately predicted human hazard. Subsequently extensive surveys of consumer and employee eye response to a variety of detergent products have been conducted by Freeburg et al. (1984, 1986-b). Results from rabbit tests using 0.01 ml of test material correlated most closely with the human eye data. This finding was supported by the results of an eye study with human volunteers (Freeburg et al. 1986-a).

ECETOC supports the view that for detergent type products the low volume method appears to be more accurate in predicting human eye responses and helps to achieve a reduction in potential animal discomfort/or suffering. In the absence of supportive human data, however, ECETOC considers that at present it is not possible to extend this conclusion to a wider range of materials.

1.3. Use of Dilutions of Test Materials.

Bell et al (1979) proposed that shampoos should be tested after dilution with water to a standard active matter content. The authors compared the relative irritancy of shampoos diluted to aqueous solutions containing approximately 20% active matter. The authors concluded that "rabbit eye testing should be regarded not as a facsimile representation of likely effects but as a means of comparing the relative eye irritancy of shampoo formulations.....".

ECETOC considers that while it may be useful for comparative purpose, this approach does not allow an assessment of the irritancy potential of the (undiluted) material to which man may be exposed.

2. Replacement by Alternative Test Systems

A review of the literature has identified several methods that employ various in-vitro test systems to predict in-vivo ocular irritancy. These techniques are summarised in Appendix 3; the test object, the procedure, and the endpoint measured are identified. The procedures can be classified into :

i) cell cultures;
ii) organ cultures;
iii) other procedures.

ECETOC has assessed their value by taking into account the extent of validation, the number of chemicals and the number of chemical classes examined. The reproducibility and limitations of each technique were also considered together with any reported correlations with the Draize test. Other recent reviews on alternative test systems include Scaife (1985) and Frazier et al. (1987).

2.1. Cell cultures. (Appendix 3-1)

Various types of cells e.g. liver fibroblasts, corneal cells etc. of human or animal origin have been cultured and treated with a range of concentrations of test material. Cell damage or death have generally been taken as the measured endpoint although there are numerous ways of assessing damage and/or death. The concentration of test material required to kill a specific proportion (usually 50%) of the cultured cells has been used as the indicator of eye irritation potential. In general LC_{50} values correlated more closely with in vivo eye irritancy data when chemically related materials were tested. The correlation was less consistent for unrelated chemicals. That there is no overall correlation is unsurprising since it is unreasonable to expect a simple value such as an LC50 obtained from an in-vitro test to reflect the complexities of an in-vivo inflammatory response. Furthermore, the physico-chemical properties of a test material (solubility in and miscibility with the culture medium) may make it unsuitable for evaluation by this approach.

2.2. Organ Cultures (Appendix 3-2)

2.2.1. Enucleated eyes. The use of the enucleated eye allows a study of the direct effect of materials on the cornea. Test materials are applied to the cornea for a predetermined time and the effects on corneal opacity and integrity of the corneal epithelium are assessed. Corneal thickness is measured objectively using a slit lamp. At the end of the experiment the morphology of the cornea can be assessed histologically.

Burton et al. (1981) examined the effects of 12 different chemicals on the cornea whilst York et al.(1982) examined the effects of contact time and concentration of a shampoo on the cornea. Burton et al. (1981) concluded that the procedure can be used to identify materials which are likely to be severely damaging to the eye (and which therefore should not be tested in-vivo). York et al.(1982) concluded that the procedure enables similar chemicals/products to be ranked in terms of potential to damage the cornea.

Price and Andrews (1985) examined 60 chemicals and Koeter and Prinsen (1985) 39 chemicals using the enucleated eye. The results from these papers suggest that a similar ranking of eye irritation can be obtained from this in vitro test and the Draize rabbit eye test.

It should be emphasised that the method cannot be used to assess effects on the conjunctiva, neither does it allow an identification of those chemicals which cause an initial pain response in the in-vivo eye test. Furthermore, since the eye is isolated, the method cannot be used to assess recovery.

2.2.2. Isolated Cornea (Appendix 3-2). Corneal tissue isolated from rabbit and bovine eye has been used to study surfactants. Changes in corneal opacity, thickness and electrical impedance have been measured. The studies have been carried out on so few materials that the value of this approach in assessing ocular irritation potential is difficult to judge.

2.2.3. Rabbit isolated ileum (Appendix 3-2). The concentration of materials required to reduce by 50% the rate of spontaneous contractions of isolated rabbit ileum has been determined and compared to ocular irritancy of the same materials. Few chemicals have been tested but a reasonable correlation with in-vivo data has been found for surfactants. There is no apparent mechanistic link for the action of chemicals on the rabbit isolated ilium and the rabbit eye.

2.3. Chorioallantoic Membrane Test (HET - CAM). (Appendix 3-3). Several variations of this technique have been described. All require that the test material, in a suitable form, is applied to the chorioallantoic membrane of a fertilized hens egg which has been incubated for 10-14 days. The immediate and subsequent effects on the membrane are noted which include : coagulation of the protein in and around the blood vessels, hyperaemia and haemorrhage, lysis of the blood vessels and lesion size. The speed of appearance and the degree of change are important for the estimation of the effects.

Several investigators have used this technique; some authors considered it to be predictive of the response of the human eye to chemicals (Kong, 1987).

2.4. Other Techniques

2.4.1. Mobility of protozoa (Appendix 3-4). The inhibition of the mobility of Tetrahymena thermophila, taken as an indication of the toxicity of the test materials, was assessed for 30 compounds. There was agreement between the ocular irritancy of rabbits (published in the literature) and the toxicity measured for over half of the 30 compounds tested.

2.4.2. Haemolysis of red blood cells (Appendix 3-4). Two studies have been reported which showed a poor correlation between in vivo ocular irritancy of surfactants and their ability to cause haemolysis of erythrocytes.

2.5. Limitations of in-vitro Models as Alternatives to the in-vivo Draize Rabbit Eye Test

- None have been sufficiently validated with a large number and variety of chemicals;
- None of these tests give information on the effects on all the components of the anterior eye;
- None allow evaluation of the reversibility of ocular lesions;
- None permit an evaluation of the discomfort or pain likely to result as a consequence of the initial exposure of the eye to chemicals;
- None permit an evaluation of systemic toxicity;
- None emulate all possible mechanisms of irritancy that may occur in vivo.

For these reasons ECETOC considers that the in vitro techniques currently available are unsuitable as a total replacement for the Draize rabbit eye test. However, in vitro techniques may have a role as screening methods to identify severe eye irritants or to rank the irritancy of materials and products of a similar type.

3. Use of Stepwise Programmes

At present there is no suitable alternative to the use of live animals for determining ocular irritation potential. However it is possible to reduce the number of animals employed and the likelihood of occurrence of pronounced responses by using stepwise procedures to identify at an early stage those materials likely to be severely irritant to the eye (Falahee et al., 1983; Jackson and Rutty, 1985. Fielder et al., 1987; OECD, 1987). The simplest stepwise procedure so far described is that in the OECD Test TG - 405 which proposes the use of a single animal test in the first instance. A typical stepwise programme is represented in Fig. 1.

ECETOC supports the use of any stepwise strategy which encourages a cautious experimental approach to the assessment of ocular hazard. Fig. 1 is representative and not exclusive and ECETOC, in supporting a stepwise principle encourages the inclusion of any relevant information that may eliminate the need for an animal test.

4. Further Work

None of the alternative techniques described above have been subjected to a thorough validation programme. Some of the techniques are currently being scientifically reviewed in the literature whilst others are at an earlier phase of validation . At the present time all of the alternatives may have a role as adjuncts to the Draize rabbit eye test but none are sufficiently well validated to replace the test completely.

The validation of any procedure needs to be thorough, logical and comprehensive. In the first instance an in-house programme is normally required using a small number/ range of chemicals which is then expanded to cover many chemical types. The in-house studies are then followed by inter-laboratory studies or ring tests to determine reproducibility of the test and also increase the number of chemical types examined. Ultimately the results of studies are published. The validation of a procedure could take many years to complete.

ECETOC considers that further validation work on in vitro tests for identifying ocular hazard is required. A comprehensive validation process is essential to define the sensitivity and specificity of any in-vitro test system, i.e. its accuracy in identifying irritant and non-irritant eye materials. It is particularly important for hazard evaluation that any in-vitro test will give no false negative results.

F. INTERPRETATION OF EYE IRRITATION TEST RESULTS

1. Introduction

Data obtained from the Draize rabbit eye test (see Chapter B) on the intrinsic irritation potential of materials are used to assess the ocular hazard to man and to meet national regulatory requirements for classification of substance as to their hazard to the eye. Methods, hazard evaluation and classification are intimately connected and whatever the reason for change (relevance to human hazard or humane use of animals) to the initial assessment of eye irritation, the consequence(s) on classification must be examined.

2. Classification

Essentially four different approaches to the classification of ocular hazard have been developed (EEC, 1983; FHSA, 1979; NAS, 1977; Kay and Calandra, 1962) some of which have been used in the international regulation of chemical products. The classification procedures are similar in that all are based on the rabbit eye model and utilise the Draize scoring system for ranking responses and none consider data from irrigation studies or the initial pain response following instillation. The scores are processed differently to decide if a test substance is irritant or not and/or to grade the severity of the irritation. In addition, the following features are of varying importance in each classification scheme :

(a) the intensity of the irritant response in different regions of the anterior eye;
(b) the "weighting" of response scores prior to determining an irritation index;
(c) the level of a response during the first three days after exposure;
(d) the (ir)reversibility of a response.

The four representative approaches to classification are detailed in Appendix 4 and their essential features are discussed below.

2.1. EEC (1983)

The EEC approach is based primarily on (a) and (c) above. In this scheme two risk phrases are identified (R 36, irritating to eyes, and R 41, risk of serious damage to eyes) and are allocated when the mean intensity of response exceeds defined levels. Scores at 24, 48 and 72 hours are combined in the calculation of the overall response. Responses after 72 hours are not considered. In addition the calculation is different depending on the number of rabbits used.

2.2. FHSA (1979)

The FHSA approach is based primarily on (a) and (c) above. In this scheme an eye irritant is one that gives a positive test (see Appendix 4). Like the EEC approach, only responses at 24, 48 and 72 hours are

considered. Unlike the EEC approach, a positive response in 4 of 6 animals at any of the three time points signifies a positive test. In addition some of the threshold scores for a positive reaction are lower than those used by the EEC. Consequently, when compared to that of the EEC, the FHSA approach is likely to classify more materials as eye irritants.

2.3. NAS (1977)

The NAS approach is based primarily on (a) and (d) above. Essentially this approach ranks irritancy in terms of reversibility within 21 days (see Appendix 4). Response intensity is also taken into account but generally the scheme assumes that the more severe the lesion the greater its duration. A major difference to both the EEC and FHSA approach is the NAS recommendation that a substance is classified according to the most severe responder in a test group. In addition, the NAS classification is the only one which separates eye irritation from eye corrosion. The latter is defined as the persistence of substantial irritation for longer than 21 days.

2.4. Kay and Calandra (1962)

The Kay and Calandra method is based on (b), (c) and (d) above. This approach to the interpretation of irritation data incorporates both intensity and duration of response up to 7 days. The maximum mean score during the first 4 days is used to derive an initial classification which is then refined on the basis of persistence of response and its intensity in individual animals (see Appendix 4).

The Kay and Calandra scheme incorporates many of the features of the previous three approaches. It is the only system which totally endorses the original Draize scoring approach (see Table 1) i.e. it is the only system which considers :

i) the area as well as the degree of opacity of the involved cornea;

ii) discharge from the eye;

iii) the differential "weights" for the scores for cornea, iris and conjunctivae.

2.5. Summary

It is apparent that there are major differencies between the four representative classification systems (see Table 3) outlined above in relation to :

(i) the use of mean or individual animals scores;
(ii) the level of response which is considered significant or "positive" in relation to irritation and;
(iii) whether (ir)reversibility is taken into account.

It is clear that these differences could lead to a different classification of hazard based on the same experimental data. Of the four systems that of the NAS is most likely to classify a chemical as an eye irritant, followed by FHSA, Kay and Calandra and the EEC.

ECETOC considers there is an opportunity for international harmonisation of regulations with respect to their methods of classifying eye irritants. It is also apparent that any alteration to existing methodologies may affect each classification scheme to different degrees. None of the above approaches to classification consider the initial pain response after instillation of test compound or the results of a complementary irrigation study (see Chapter D).

3. Consequences of Modifying the Draize Eye Test and Alternative Approaches to Assessing Eye Irritation

3.1. Modifications to the Draize Eye Test

The low volume method has been reported to lead to both a more humane test (since responses are generally attenuated) and to a closer correlation with human eye response (see Chapter E). This method may be acceptable for either of the above reasons but the consequence on classification is different in each case.

If it can be shown that the low volume method coupled to current classification systems is more predictive of the likely human response then obviously no alteration to current classification systems will be necessary. In contrast, if the low volume approach is introduced on humane grounds alone then a realignment of existing classifications of irritancy with response may be necessary (see Appendix 5) to maintain current chemical/product classifications. For example, Arthur et al.(1986) proposed a modification to the Kay and Calandra scheme to achieve a comparable categorisation of irritancy using data from a 50µl compared to a 100µl volume test. Additional comparative data of this type would be needed to define how to approach any necessary realignment of classification criteria to modified test procedures.

Any changes to existing test methods which lead to more lenient classification of eye irritants would result in the need to reassess existing chemicals and thus to an increased use of animals.

3.2. In-Vitro Replacement Tests.

In-vitro models do not provide data comparable to that from an in-vivo test and cannot be used with current classification systems. Thus, adoption of in-vitro tests for predicting ocular irritation would require their alignment with existing classification criteria. This alignment would have to be achieved by comparing the relevant in-vitro parameter(s) of effect with an in-vivo hazard category using materials of known irritation potential in-vivo. The in-vivo irritation potential would need to be determined from the Draize rabbit test since there are insufficient reports of human ocular response to exposure to chemicals (see Chapter D).

The interpretation of any in-vitro test validated against the Draize model would need to be re-evaluated if the Draize model was modified. Freeberg et al.(1986a-b), for example, have suggested that a low volume test (0.01 ml) should be used to judge new eye irritation methods since the low volume test has been claimed to be predictive of human response.

3.3. Stepwise Approaches

The implementation of stepwise or hierarchical approaches to the assessment of eye irritation has been proposed to eliminate the possibility of severe responses (see Chapter E). As a consequence a chemical may be assumed to be a severe eye irritant, although a complete rabbit test with 3 animals may not have been performed.

In contrast, ECETOC considers it is unacceptable to classify a chemical as non-irritant to the eye on the basis of ancilliary data in a stepwise approach. The weight of evidence must always be to prove the absence of hazard in an animal test completed to protocol.

The adoption of certain criteria in a stepwise approach may lead to an overestimation of eye irritation potential. For example, chemicals which are severe skin irritants are not necessarily eye irritants (see Chapter C). The acceptance of all severe skin irritants as eye irritants may lead to over-classification and will not permit the separate classification of materials which are irritant and those which are more severely irritant to the eye.

In contrast to the EEC (1984) those regulations which are based on the NAS scheme (1977) require the separate classification of eye - corrosive and eye-irritant chemicals. Under these regulations, the decision not to test for eye irritation potential may necessitate the classification of a material as corrosive to the eye. Such a situation may inhibit the utilisation of stepwise approaches in order to avoid overlabeling.

ECETOC questions the need to distinguish experimentally in a rabbit eye test between a severe irritant and an eye corrosive chemical. Adequate preventive and full protective measures should be taken to exclude either category of material from human eye contact.

G. RECOMMENDATIONS

1. There is a need to harmonise existing guidelines on test methods with respect to the number of animals required. There is little justification for using more than three rabbits in the Draize test or the routine use of extra animals for irrigation studies.

2. In order to optimise a humane approach to the determination of ocular hazard using a rabbit eye test it is recommended that a stepwise test strategy is always used; in addition the low volume method should be further validated.

3. The response of the animals when the material is first instilled into the eye should be considered in the overall classification of a chemical as an eye irritant. The rabbit eye test should not be used to distinguish between severe eye irritants and corrosive chemicals on the basis of days for a lesion to recover.

4. The continued development and validation of in vitro tests should be encouraged so that their potential in prescreening and as alternatives to the Draize test can be evaluated.

5. There is a need to harmonise systems for classifying ocular hazard at an international level; this will require the collaboration of relevant regulatory bodies. At present the existence of a number of different classification schemes may result in different classifications of hazard from the same experimental data. It should be realised that any change in test methods will have an impact on the existing hazard classification schemes.

6. More information should be gathered about the effects of chemicals on the human eye. These data are necessary to assess fully the relevance of the present methods and classification systems for evaluating ocular hazard to man.

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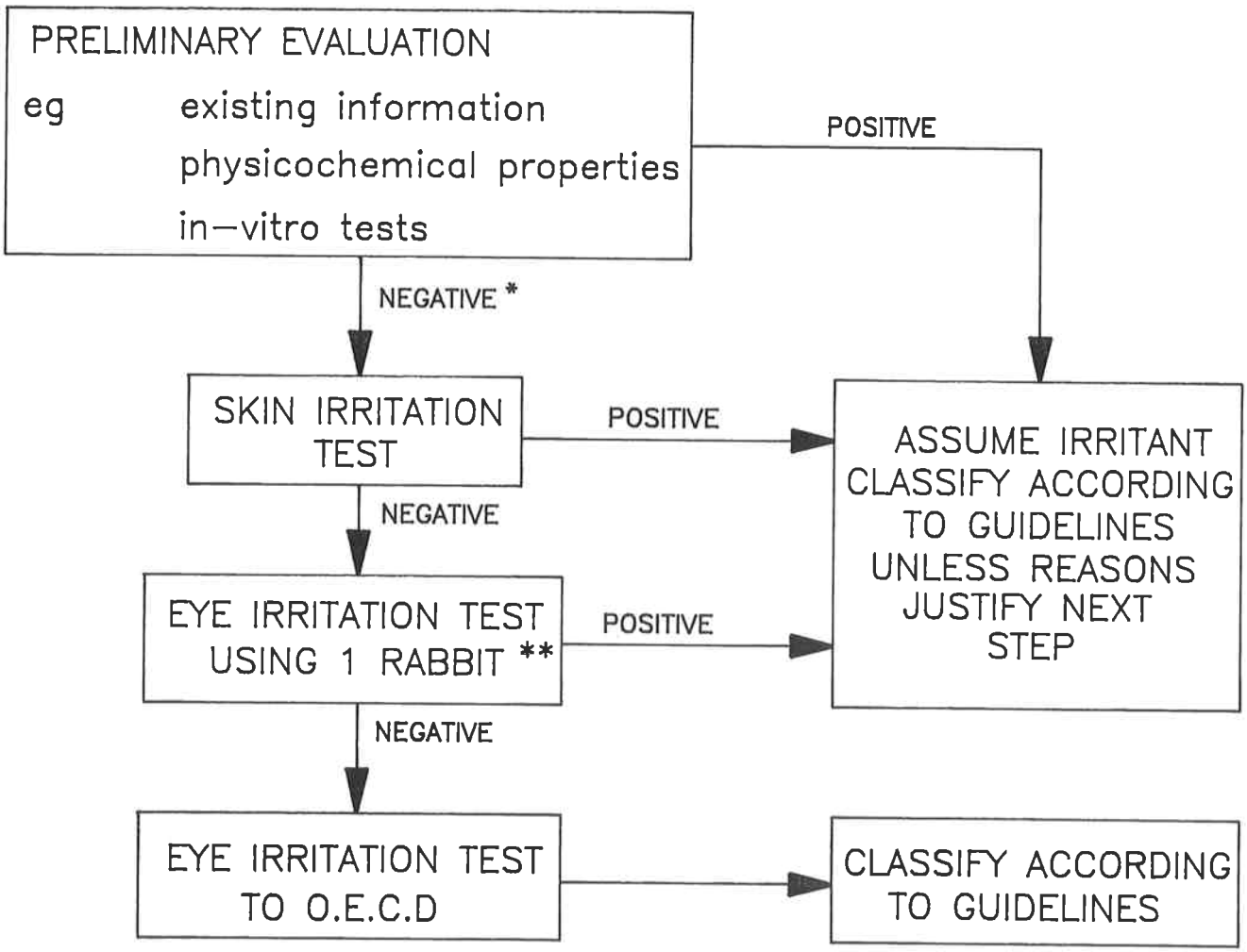
FIG.1. AN EXAMPLE OF A STEPWISE STRATEGY FOR ASSESSING OCULAR IRRITATION



* *The negative and positive criteria are defined by either (a) the experimentalist or (b) international guidelines*

** *At the in-vivo level the use of diluted material, a reduced volume or anaesthesia may be appropriate as part of the stepwise policy for assessment of ocular irritation.*

TABLE 1

SCALE FOR SCORING OCULAR LESIONS,

1) CORNEA

(A) Opacity-degree of density (area most dense taken for reading)
- No opacity 0
- Scattered or diffuse area, details of iris clearly visible 1
- Easily discernible translucent areas, details of iris slightly obscured 2
- Opalescent areas, no details of iris visible, size of pupil barely discernible 3
- Opaque, iris invisible 4

(B) Area of cornea involved
- One quarter (or less), but not zero 1
- Greater than one quarter, but less than half 2
- Greater than half, but less than three quarters 3
- Greater than three quarters, up to whole area 4

Score equals A x B x 5 Total maximum = 80

2) IRIS

(A) Values
- Normal 0
- Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) 1
- No reaction to light, hemorrhage, gross destruction (any or all of these) 2

Score equals A x 5 Total maximum = 10

3) CONJUNCTIVAE

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
- Vessels normal 0
- Vessels definitely injected above normal 1
- More diffuse, deeper crimson red, individual vessels not easily discernible 2
- Diffuse beefy red 3

(B) Chemosis
- No swelling 0
- Any swelling above normal (includes nictitating membrane) 1
- Obvious swelling with partial eversion of lids 2
- Swelling with lids about half closed 3
- Swelling with lids about half closed to completely closed 4

(C) Discharge
- No discharge 0
- Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
- Discharge with moistening of the lids and hairs just adjacent to lids 2
- Discharge with moistening of the lids and hairs, and considerable area around the eye 3

Score equals (A + B + C) x 2 Total maximum = 20

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible = 110.

(Adapted from Draize, 1959)

TABLE 2

CRITICISMS OF THE DRAIZE/FHSA TEST FOR EYE IRRITATION
(adapted from Fallahee et al. 1981)

Methodology	
Assessment of reaction is influenced by the test group size and the length of the observation period	Weltman et al (1965)
Subjective nature of the scoring system	Buehler (1974) Heywood and James (1978)
Interlaboratory variability in tests with identical materials	Russell and Hoch (196 Rieger and Battista (1964) Weil and Scala (1971)
Difficulties in interpretation of test results	Kay and Calandra (196 Ballantyne and Swanst (1977)
Inability to correlate active inflammatory signs with permanent structural change	Aronson (1975)
Relevance to man	
Discrepancies in response of the rabbit and human eye : test is performed in rabbits but results are applied to humans	Buehler (1974) Marzulli and Simon (1971)
Method of exposure to test agent not comparable with means of human accidental exposure in the case of aerosol, powders, and granular substances.	Beckley (1965-b)
Volume of materials as tested produces exaggerated results in the rabbit eye compared to findings in man	Griffith et al (1980)
Humane Considerations	Rowan (1980, 1981) Harriton (1981)

Summary of Criteria Relevant to Classifying a Substance as "Irritant".

Classification System	Basis of Classification	Response Intensity	'Weighting' of Response Intensity	Level of Response During First Three Days	Reversibility of Response
EEC (1983)	Mean response of 6 animals; worst 2 of 3 animals	Considered	Not considered	Considered	Not considered
FSHA (1979)	If 4 of 6 animals 'positive'	Considered	Not considered	Considered	Not considered
NAS (1977)	Consider most severe responder of group	Considered but less important than reversibility	Not considered	Longer time-scale considered	Considered (up to 21 days)
Kay and Calandra (1962)	Mean response and also individual animal scores if response lasts longer than 7 days	Considered	Considered	Considered in conjunction with longer time-scales	Considered (up to 7 days)

APPENDIX I : Test Guidelines for Eye Irritation Testing

OECD (1987)	OECD (1981)	EEC (1984)	AFNOR (1982)	EPA/TSCA (1985)	FHSA (1973)	EPA/FIFRA (1984)	UK CONTROL OF PESTICIDES (1986)	JAPAN MAFF (1985)
SPECIES								
Healthy Adult Albino Rabbit recommended.	-	-	Male Albino Rabbit approx. 2.5 kg	Justification needed for use of other mammalian species	Albino Rabbit	Justification needed for use of other mammalian species	Rabbit recommended	Young Albino Rabbit
GROUP SIZE								
1. Test on one animal to be considered if marked effects are anticipated. If severe irritant or corrosive further testing may be unnecessary.	-	No ref. to one animal test	No ref. to one animal test	See below	No ref. to one animal test	See below	Using one rabbit - if Strongly irritant study of effect of irrigation should be assessed	No ref. to one animal test
or								
2. At least 3 animals. Extra may be required to clarify equivocal responses.	-	-	6	At least 6 Justification required for using fewer	At least 6 but depending on result may require 2 more groups of 6 i.e. 18 total	At least 6 Justification required for using less fewer	Substances not strongly irritant - 2 further animals required	At least 6
EXCLUSIONS								
1. Materials with pH≤2 and ≥11.5. Buffer capacity should be considered.	Strongly acidic or alkaline materials e.g. e.g. pH≤2 and ≥11.5	Strongly alkaline or acidic materials	-	-	no exclusions specified	- -	Testing in undiluted form is unnecessary -	-
2. Severe skin irritants and corrosives.	-	-	No ref. to irritants	-				No ref. to skin irritation
3. Results from well validated alternatives demonstrating potential corrosive or severe irritant.	No ref. to alternatives	No ref. to alternatives	No ref.	No ref. to alternatives	No ref. to alternatives		In-vitro alternatives not completely accepted	
DOSE LEVELS								
Liquids : 0.1 ml (undiluted)	-	-	-	-	-	-	0.01 ml or 10 mg on corneal surface offered as alternatives	-
Solids and Pastes : 100 mg or 0.1 ml	-	-	100 mg for paste, powder, solid.	-	-	-		-
Aerosols : spray from 10 cm for 1 sec.	-	No ref. to	No ref. to	-	No ref. to	-	No reference to	No ref. to

ADDITIONAL TREATMENT 1. Local anaesthetic may be used.	No ref. to use of anaesthetics	-	No ref. to use of anaesthetics	No ref. to use of anaesthetics	No ref. to use of anaesthetics.	-	No ref. to use of anaesthetics	No ref. to use of anaesthetics
2. Wash after 24 hrs if appropriate.	-	-		-	After 24 hrs with saline		Not specified See below	Eyes should not be washed for 24 hrs
3. Using 3 animals. 0.5 min. after instillation of test material. Wash eyes for 0.5 min.	3 animals wash eyes for 5 min., 4 sec. after instillation of test mat. 3 animals wash eyes for 5 min., 30 sec. after instillation of test mat.	3 rabbits - irrigated after 4 secs 3 rabbits after 30 secs. Irrigate for 4 mins.	If irritant - test in 6 more rabbits, with irrigation 60 secs after instillation of test material	No ref. to irrigation	No ref. to irrigation	No ref. to irrigation	200 ml tap water for 2 mins No of animals unspecified	If irritation persists to 72 hrs irrigation efficiency should be checked with 3 animals 2-3 min after instillation
OBSERVATION Duration not fixed rigidly but should be long enough to determine (ir)reversibility of effects. Not normally more than 21 days.	-	-	Duration up to 14 or 21 days to determine (ir) reversibility of effects	Duration not fixed rigidly but should be at least 72 hrs. Not normal to exceed 21 days.	Implied that duration need not exceed 72 hrs	Duration not fixed rigidly but should be at least 72 hrs. Normally need not exceed 21 days.		If no irritation at 72 hrs, the study may be terminated. Observation normally need not exceed 21 days.
SCORING At observation times 1, 24, 48 and 72 hrs.	-	-	-	-	Ocular reactions graded at 24, 48, 72 hrs	-	- Intermediate times may be necessary	-
Score responses on following scale						-	-	-
Cornea 0-4	-	-	-	-	-	-	-	-
Iris 0-2	-	-	-	-	-	-	-	-
Conjunctiva (redness) 0-3	-	-	-	-	-	-	-	-
Conjunctiva (chemosis) 0-4	-		-	-	-	-	-	-

- signifies that requirement is same as that in Test Guideline 405 (OECD 1987).

APPENDIX 2

NUMBERS OF REPORTED HUMAN EYE ACCIDENTS, AND THE RELATIONSHIP BETWEEN THE PREDICTED HAZARD EVALUATION AND THE REPORTED HUMAN RESPONSE.

No. Incidents	No. Chemicals	Physical state of Chemical			No. Incidents listed according to hazard evaluation		No. Incidents listed according to severity of human response as number of days for response clear			
		L	S	V	Hazard Category*	No. Incidents	<3 days	3-7 days	7-21 days	>21 days
171	134	159	8	4	severe	60	43	4	11**	2**
					moderate	40	34	5	1	-
					mild	39	37	1	1***	-
					non irritant	4	4	-	-	-
					not known	28	28	-	-	

L = liquid S = solid V = vapour

* Hazard evaluation : the hazard category has been derived from literature references and animal studies. It is, therefore, arbitrary but the terms and categories used comply as far as possible with the scheme of Kay and Calandra

** Chemicals strongly acidic or basic.

*** Pesticide formulation : ocular symptomology difficult to dissociate from reported and prolonged systemic symptomology.

The information presented in this Appendix was obtained from Occupational Health records of ECETOC member companies and shows incidents of human eye accidents collected over an approximately 2-year period (1984-1986) and involving industrial and agrochemicals. The incidents have been subdivided on the basis of the hazard category of the chemicals and compared with the severity of the human response which is based on "days for any effect to clear".

Any analysis of the tabulated data must be viewed against the following :

1. The data represent what has been submitted to ECETOC and should be considered as a selected rather than a representative sample of adverse effects of chemicals in the human eye;

2. Only one case was reported in which no injury followed exposure. The data set undoubtedly probably represents under-reporting of exposures which failed to produce human response;

3. The "days to clear" have been estimated from the submitted medical records i.e. a response was assumed to be negligible/recovered if a patient ceased to report for medical attention;

4. In all cases it was not possible to estimate the amount of material entering the eye;

5. No information was available on the safety precautions in operation at the time of the human eye exposure;

6. There is no available information on immediate first-aid measures and subsequent medical treatment.

APPENDIX 3

Alternative in vitro Methods

APPENDIX 3:

ALTERNATIVE IN VITRO METHODS

3.1: Cell Cultures (Cytotoxicity Tests)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
Human hepatoma cells (Hep G2) Murine fibroblasts (BALB/c 3T3), Chinese hamster lung fibroblasts (V 79), Murine macrophage cultures, Epithelial rabbit cornea cells	1) Pre-incubation 24 h 2) Treatment with test-compound and incubation for 24 h 3) Cells in new normal medium were allowed to replicate for 7 days to form colonies 4) Cultures fixed, stained and colonies counted 5) Results are expressed as per cent colony formation of untreated controls	Alcohols, ethers, esters, ketones, acids, amides, detergents, benzalkonium chloride, benzethonium chloride Tween (34 substances)	The highest tolerated dosages determined for each test compound were ranked to each other and to published rabbit ocular irritancy data. "Good agreement between all 5 methods" and "well in ranking" to ocular irritancy Borenfreund and Borrero (1984)
BALB/c 3T3 mouse fibroblast cells	1) Pre-incubation 24 h 2) Treatment with test compound and incubation for 24 h 3a) Scoring morphologically for cytotoxic effects 3b) Determination of Neutral Red uptake spectrophotometrically	Benzalkonium chloride, benzethonium chloride, Triton X-155, sodium lauryl sulfate, Tween 80, TEA-laurylsulfat (6 substances)	The observed ranking in both evaluations (cytotoxic effects and Neutral Red uptake) are in "agreement" with in vivo (Draize test) observations Borenfreund and Puerner (1985)
Cells from the corneas of normal rabbit eyes (SIRC rabbit corneal cells)	Reduction of cloning efficiency 1) 400 cells pre-incubation 18 h 2) Treatment for 1 h with test substances in different concentrations 3) Incubation for 7 days to form colonies before determining the mean per cent survival in relation to negative controls and LC50	13 surfactants (5 cationics, 3 anionics, 2 amphoterics, 3 nonionics) 6 shampoos	Rank correlation analysis indicated a correlation coefficient of 0.90 North-Root et al. (1982) North-Root et al. (1983) 100 % agreement North-Root et al. (1985)

APPENDIX 3: (continued 2)

3.1: Cell Cultures (Cell Detachment, Growth Inhibition and Cloning Efficiency Test)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
1. Baby hamster kidney fibroblasts (BHK-21/C13) 2. Early (Keller) and late (MRC-5) passage human fibroblasts	3 parameters measured 1. Cell detachment assay 24 h pre-incubation 4 h exposure to the test substance 2. Growth inhibition 24 h pre-incubation 48 h exposure to the test substance Cells counted before and after incubation 3. Cloning efficiency 7 days exposure to the test substance cells fixed and stained with Giemsa Cell detachment assay and cloning efficiency number of colonies expressed as percentage of control (IC50)	57 chemicals of various classes (inorganic and organic metal salts, solvents, detergents, reagents, drugs)	The ranking order resulting from in vitro data correlated better with threshold limit values for human workroom air (TLV/TWA) than with LD50-values (rat, oral). Correlation with data from Draize skin and eye irritation tests were not determined since the available in vivo values were derived using various different scoring systems. But the in vitro data were more than 80 % preditive of in vivo classification (skin and eye irritation) when used to divide the chemicals into three crude classes (non, mild to moderate, strong irritant or corrosive) Reinhardt et al. (1985)
		3 endogenous chemicals (glutathione, l-methionine, l-cysteine HCl) and 4 organo tin compounds	Correlation was not calculated, no in vivo data available, but both assays rank all compounds tested in the same sequence of toxicity as that known from in vivo studies Reinhardt et al. (1982)

APPENDIX 3: (continued 3)

3.1: Cell Cultures (Neutral Red uptake, 51Cr-Release Test)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
BALB/c-3T3 cells (mouse fibroblasts)	Uptake of Neutral Red (NR) as a measure of the toxic effects of substances 1) Pre-incubation 24 h 2) Test samples added to the semi-confluent cultures incubation time 24 h 3) Test samples removed after 3 h incubation 4) Determination of uptake of NR NR concentration in extraction medium is determined spectrophotometrically, result expressed as a percentage of the control values	Benzalkonium chloride, benzethonium chloride, Triton X-155, sodium lauryl sulfate, Tween 60, TEA-laurylsulfate (6 substances)	Neutral Red uptake in agreement with in vivo (Draize-Test) observations Borenfreund and Puerner (1985)
		Different chemicals, alcohols, surfactants and others (35 substances)	When used as screening test reasonable correlation depending on the class of chemical tested Künstler et al. (1986-b)
		9 test compounds (different chemical substances with eye irritancy classification from none, mild, moderate to severe)	NR-uptake and eye irritancy (Draize-Test) showed "a good agreement" Hockley and Baxter (1986)
Primary corneal epithelial cell cultures from rabbits SIRC-cells, YAC-cells, P 815-cells tumour cell line P 815, YAC-1, SIRC-cells	1) Cells radiolabelled with ^{51}Cr for 2 h 2) Exposure of radiolabelled cells to serial two fold dilutions of test material for 4 h 3) Centrifugation of the microtitre plates 4) Determination of the ^{51}Cr in the supernatants with a gamma counter CD50 represents the concentration of test material which caused release of 50 % of radiolabelled cells	6 surfactants (benzalkonium chloride, 2-alkylethoxylated sulfates, sodium dodecyl sulfate, coconut soap, Polysorbate 20)	"Nice correlation" between in vitro and in vivo data Shadduck et al. (1985-a)

APPENDIX 3: (continued 4)

3.1: Cell Cultures (Cytotoxicity Test, Cell Membrane Assay)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
Human epithelioid cell lines (Hela and HEp2)	Cell-membrane integrity assessed by using fluorescein diacetate/ ethidium bromide. Loss of this fluorochromasia correlated with other indicators of cell damage	12 surfactants	Variable correlation with in vivo data for anionic and cationic surfactants Scaife (1982)
	Assessment of cell-membrane integrity by measurement of the release of alkaline phosphatase. Data assessed by determination of EC50 values	4 surfactants	"Good correlation" between in vitro data and rabbit eye irritation data Scaife (1985)
Mouse LS-cells derived from NCTC L 925 mouse fibroblasts	50 % cell death (CD50) after exposure to test samples for 4 h	11 detergent-based commercial products	With one exception, reasonable correlation between in vivo and in vitro data Kemp et al. (1983)

APPENDIX 3: (continued 5)

3.1: Cell Cultures (Protein Cell Growth Assay, Uridine Uptake Assay)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
Mouse fibroblasts BALB/c-3T3-cells and other cells	Inhibition of protein production after incubation with the test substances is measured 24 h incubation in growth medium together with medium containing test materials in various concentrations	25 chemicals (alcohols, ethers, esters, ketones, amids, acids, detergents)	Ranking correlated well with published data of Draize test Shopsis and Sathe (1984)
		19 chemicals (alcohols etc.), 17 surfactants	Cytotoxic morphological changes correlate "very well" with ocular irritancy Shopsis et al. (1985)
	1) Cell toxicity assessed by phase contrast microscopy 2) Cell protein determined by rapid semi-automated method with the Bio-Rad dye reagent 3) Results: ug protein/well expressed as percentage of the cell protein in untreated cells	9 alcohols	Good agreement with the relative irritancies in Draize rabbit eye test results Borenfreund et al.(1983)
		39 substances (chemicals, antimicrobiol agents, cosmetics)	Bad correlation, cell cultures are to sensitive Triemer et al. (1986)
BALB/c 3T3-cells and other cells	Inhibition of uridine uptake is measured. 1) Cells exposed to various concentrations of test samples for 4 h 2) ^{3}H-uridine uptake determined over subsequent 15 min period Toxicity assessed as the concentrations of test material (ug/ml) required for 20 %, 50 % and 80 % inhibition	25 chemicals (alcohols, ethers, esters, ketones, amids, acids, detergents)	Ranking correlated well with published data of Draize test Shopsis and Sathe (1984)
		19 chemicals (alcohols, etc.) 17 surfactants	Uridine uptake inhibition correlated well with Draize data, when the two groups are evaluated separately Shopsis et al. (1985)
		9 alcohols	Good agreement with in vivo results Borenfreund et al. (1983)
		14 different chemicals, alcohols, surfactants	Used as screening test. More or less good correlation depending of the chemical class of tested substances Künstler et al. (1986-a)

APPENDIX 3: (continued 6).

3.2: Organs and Organ Cultures (Enucleated Rabbit Eyes, Isolated Rabbit Cornea)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
Enucleated rabbit eyes maintained in saline perfusion chambers	1) Corneal thickness is determined with a slit lamp biomicroscope fitted with depth measuring attachment 2) Solid samples (50 mg) are placed on the corneal surface. Liquids are applied on the upper margin of the cornea 3) Contact time ranged from 10 sec to 5 min	11 substances (sodium hydroxide, acetic anhydride, formaldehyde, allyl alcohol, n-butanol, ethanol, acetone, QAV, toluene, propylene, glycol, glycerine)	Results of in vitro tests "correlated broadly" with own in vivo data and data recorded in the literature Burton et al. (1981)
	4) Corneal swelling, gross corneal changes and histological integrity of epithelium evaluated up to 4 h after treatment	11 substances and 1 shampoo (100 and 10 %) 10, 30 sec., 2 and 5 min contact time	"Reasonable" correlation between in vitro and in vivo results York et al. (1982)
		60 chemicals (25 industrial chemicals, 18 formulated agrochemicals, 3 unformulated agrochemicals, 14 formulated lubricating oils)	The rapidity and degree of corneal swelling were related to in vivo irritancy potential Price and Andrews (1985)
		39 substances (solvents like acetone, ethanol, alcohols, other substances)	Correlation between in vitro and in vivo results for 82 % of all substances Koeter and Prinsen (1985)
Isolated rabbit cornea	Changes in the electrical impedance across the isolated cornea were measured in the presence and absence of various anionic detergents	6 anionic surfactants	The rate of change of impedance decreased with an increase in alkyl chain length for sodium alkyl sulphates. Results correlated with in vivo ocular irritation test results Scaife (1985)

APPENDIX 3: (continued 7)

3.2: Organs and Organ Cultures (Corneal Opacity, Rabbit Isolated Ileum)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
Bovine cornea	1) Cornea bathed in Tyrode solution with or without test substance at 32° C 2) Measurement of corneal thickness or opacity with a special slit lamp or opacitometer 3) Concentration effect curves are drawn	Tetracaine, benoxinate, benzalkonium chloride, sodium decyl sulfate, sodium lauryl sulfate	No in vivo data available Andermann and Erhart (1983) No comparison Muir (1984-a)
		8 surfactants and 5 industrial chemicals	Corneal opacity in vitro correlated well with published in vivo data Muir (1985)
		Several concentrations of sodium lauryl sulfate	Opacity increased with increasing concentrations of NaLS Haruyoshi Igarashi (1986)
Isolated rabbit ileum	Influence of test substance of spontaneous concentrations of isolated ileum 1) Ileum set up under standard conditions in an organ bath 2) Spontaneous contractions are recorded using an oscillograph 3) Chemicals added cumulatively at 10 min intervals 4) Toxicity (EC50) assessed as the concentration which blocks 50 % of spontaneous activity	8 surfactants	Good correlation between EC50 and in vivo data Muir et al. (1983)
		4 antidandruff shampoos, 4 adult shampoos, 4 baby shampoos	Correlation with in vivo data Muir (1983-a)
		12 substances (alcohols, aldehydes etc.)	EC 50-values correlate with in vivo irritancy data Muir (1983-b)
		6 surfactants (3 sulphates, 3 bromides	Good correlation with in vivo data Muir (1984-b)
		5 antidandruff, 7 adult, 11 baby shampoos	EC50 for antidandruff and adult shampoos "correlated well" with ocular irritancy for 20 % dilution, but correlation was poor for baby shampoos Muir et al. (1986)

APPENDIX 3: (continued 8)

3.3: Hen's Egg Chorioallantoic Membrane (CAM) (HET-CAM-Assay or Chorioallantoic Membrane Test)

Test object	Testing procedure and evaluation	Tested substances	Correlation to *in vivo* data (Draize Test rabbit eye)
Fertilized hen's egg	1) Dilutions of test substances applied to CAM of 14 day hatched hen's eggs 2) Necrotic areas measured after 48 - 72 h, and lesions discribed 3) Data evaluated on size and nature of lesions, with thresholds for "positive" reactions	12 household preparations	Ranking correlation coefficient r = 0.72 Leighton et al. (1985)
		9 chemicals with known eye irritancy potential *in vivo* selected from published data	For 4 of the 9 chemicals the response in the CAM was considered to predict the in vivo activity. The CAM-test is not suitable for predicting *in vivo* irritant potential of substances for the conjunctiva. Others do not consider it to be a relevant predictive method of irritation Lawrence et al. (1986)
	1) 0.2 ml of test substances applied to the CAM on day 10 of incubation 2) 72 hours later, nature of lesions assessed (surface, blood vessels, colour)	39 substances (chemicals, surfactants, antimicrobial agents, cosmetics) with known irritancy potential from harmless to severe	CAM-test is more sensitive than rabbit eye. Good correlation for slight or strong irritants. Correlation is less good for moderate irritants. Triemer et al. (1986)
	1) 0.1 ml of test material is applied to the membrane on day 12. 2) The degree of injection and haemorrhage of blood vessels is scored at 0.5, 2, 5 and 10 min.	30 chemicals (industrial chemicals, oils, formulated pesticides)	23 chemicals were correctly identified as irritant or non irritant; five false positives and two false negatives. Ranking of intensity of CAM response did not correlate with the severity of *in vivo* effects. Price et al. (1986)

APPENDIX 3: (continued 9)

3.3: Hen's Egg Chorioallantoic Membrane (CAM) (HET-CAM-Assay or Chorioallantoic Membrane Test)

Test object	Testing procedure and evaluation	Tested substances	Correlation to *in vivo* data (Draize Test rabbit eye)
Embryonated chicken eggs	1) 0.2 ml or 0.1 g test substance applied onto the CAM on day 10. Solids rinsed after 20 sec 2) 0.5, 2 and 5 min after treatment blood vessels assessment of irritant effects (hyperaemia, haemorrhage, coagulation)	3 vehicles, 7 antimicrobial agents, 11 oxidation dyes and 8 commercial shampoos	"Good correlation" between CAM irritation and Draize test results for all tested classes of compounds Lüpke (1985-a, b)
		About 190 substances and formulations	"Good correlation" Lüpke (1986) "High reproducibility" in HET-CAM-tests between different investigators and laboratories Lüpke and Kemper (1986)
	1) 300 ul of test material applied to CAM on day 10 2) Reactions assessed continuously for 5 min after application	13 chemicals (alcohols, surfactants, and others)	The results of HET-CAM-Test are in "good accordance" with results from *in vivo* studies Künstler et al. (1986-b)
Embryonated chicken eggs	1) On day four 1.5 - 2 ml of albumin are withdrawn to create an enlarged airspace for development a CAM 2) On day 14 a teflon ring is placed on the CAM and the test material is placed into the inner area of the ring 3) On day 17 of incubation the lesion size of the CAM is evaluated	42 test substances of diverse nature (water soluble, lipophilic and particulate materials)	31 of the 42 samples were eye irritants as well *in vivo* as *in vitro* tests, 8 were false positive in CAM assay, 3 were negative in both (rabbit eye assay and CAM assay) Kong (1987)

APPENDIX 3: (continued 10)

3.4: Other Procedures (Tetrahymena Test and Erythrocyte Haemolysis Test)

Test object	Testing procedure and evaluation	Tested substances	Correlation to in vivo data (Draize Test rabbit eye)
Tetrahyma thermophila	Inhibition of mobility of a protozoa 1) Test samples are diluted in filtered MM2 medium and added in equal volume to the Tetrahyma suspension 2) Normal mobility pattern of the organisms is quantified. 3) End-point is the highest tolerated dose (HTI) allowing at least 90 % of cells to retain normal mobility	8 alcohols, 6 ethers, 2 ketones, 3 acids, 4 bases, 5 salts, formaldehyde, dimethyl sulfoxide	In 58 % of tests, results were the same as literature reports of in vivo testing. More severe reaction with T. thermophila in 27 % of the tests and a less severe reaction in 15 % of the tests. Silverman (1983)
Bovine and rabbit erythrocytes	Dose response curves were constructed for each surfactant enabling calculation of the concentration required to produce 50 % haemolysis (EC50-haemolysis)	8 anionic surfactants, 4 cationic surfactants	Haemolytic potency in vitro "failed to correlate" with in vivo rabbit eye irritancy Muir et al. (1983)
		16 anionic surfactants	No correlation Kästner and Frosch (1981)

APPENDIX 4 - APPROACHES FOR CLASSIFICATION OF OCULAR HAZARD

EEC Regulations (1983)

Ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours and correspond to the following values :

- The mean value of the scores for each type of lesion, calculated over all the animals tested, is one of the following :

	R36 Irritant to the eye	R41 Risk of serious damage to the eye
cornea opacity	two or more	three or more
iris lesion	one or more	1.5.* or more
redness of conjunctivae	2.5 or more	
oedema of conjunctivae (chemosis)	two or more	

(* two if three animals used).

- or, in the case where the Annex V test has been completed using three animals, either cornea opacity, iris lesion, redness of conjunctiva or oedema of conjunctiva (chemosis) equivalent to a mean value such as is quoted above, but calculated for each animal separately, has been observed in two or more animals.

In both cases all scores at each of the reading times (24, 48 and 72 hours) and for an effect should be used in calculating the respective means values.

FHSA Regulations (1979)

Any responses of the following intensity (or greater) at any reading (24, 48 or 72 hours are considered "significant" and thus represent a "positive" reaction in an animal:

Cornea	> 1
Iris	> 1
Conjunctiva	
Redness	> 2
Chemosis	> 2

The test is "positive" if a "positive" reaction occurs in four or more animals of six animals and the substance is classified as an eye irritant. (Code of Federal Regulation, 1979).

NAS Scheme (1977)

Substances are classified on the basis of the following criteria :

Inconsequential or complete lack of irritation : Exposure of the eye to a material under the specified conditions causes no significant ocular changes. No staining with fluorescein can be observed. Any changes that occur clear within 24 hr and are no greater than those cause by isotonic saline under the same conditions.

Moderate irritation : Exposure of the eye to the material under the specified conditions causes minor, superficial and transient changes of the cornea, iris or conjunctiva as determined by external or slit lamp examination with fluorescein staining. The appearance at the 24h or subsequent grading of any of the following changes is sufficient to characterise a response as moderate irritation opacity of the cornea (other than a slight dulling of the normal luster), hyperaemia of the iris, or swelling of the conjunctiva. Any changes that are seen clear within 7 days.

Substantial irritation : Exposure of the eye to the material under the specified conditions causes significant injury to the eye, such as loss of the corneal epithelium, corneal opacity, iris, (other than a slight

injection), conjunctivae, pannus, or bullae. The effects clear within 21 days.

Severe irritation or corrosion : Exposure of the eye to the material under the specified conditions results in the same types of injury as in the previous category and in significant necrosis or other injuries as in the previous category and in significant necrosis or other injuries that adversely affect the visual process. Injuries persist for 2 days or more.

The NAS recommends that a substance is classified according to the most severe responder in a test group.

Kay and Calandra (1962)

This procedure involves the production of an ocular irritation index based on an average of "weighed" Draize scores. This index is then modified by consideration of other factors such as recovery over 7 days and individual animal scores. The AFNOR assessment of eye irritation is based on the Kay and Callandra scheme.

APPENDIX 5

COMPARISON OF RESULTS FROM CLASSICAL DRAIZE TEST AND LOW-VOLUME TEST

The information used in this Appendix were provided by companies involved in the Task Force and also include those chemicals assessed by Griffith et al. (1980).

Seventy test materials were assessed for irritancy potential in the rabbit eye at dose volumes of 100 and 10 µl. The data are also interpreted against classification criteria of the EEC and the "pass-fail" criteria of FHSA (see chapter F). The data were generated by this Task Force. The test material were :

Substances		Preparations	
chemicals	: 14	general purpose cleaners	: 21
anionic surfactants	: 11	fabric rinse conditioners	: 6
cationic surfactants	: 4	fabric washing products	: 5
amphoteric surfactants	: 4	hair conditioners	: 3
surfactant mixtures	: 2		
	---		---
	35		35

Application of classification criteria gave the following results : -

	100 µl				10 µl			
	Classified as "irritant" by		Classified as "non-irritant" by		Classified as "irritant" by		Classified as "non-irritant" by	
	EEC	FHSA	EEC	FHSA	EEC	FHSA	EEC	FHSA
chemicals	6	7	8	7	3	6	11	8
surfactants :								
anionic	2	5	9	6	1	2	10	9
cationic	2	3	2	1	0	0	4	4
amphoteric	1	1	3	3	0	1	4	3
mixtures	1	1	1	1	0	1	2	1
general purpose cleaners	2	13	19	8	0	11	21	10
fabric rinse conditioners	3	6	3	0	0	2	6	4
fabric washing products	0	2	5	3	0	0	5	5
hair conditioners	1	2	2	1	0	0	3	3
	18	40	52	30	4	23	66	47

These results show that :

(i) At both test volumes more test materials are irritant by the FHSA criteria than are classified by EEC. FHSA criteria are thus more stringent.

(ii) a reduction in test volume from 100µl to 10 µl resulted in :
FHSA - 17 of 40 substances being "declassified "to non-irritant".
EEC - 14 of 18 substances being "declassified "to non-irritant".

APPENDIX 6 : MEMBERS OF TASK FORCE

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M. YORK	UNILEVER (Sharnbrook)
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Appendix 7 : Members of ECETOC Scientific Committee

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